ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

REVIEW ARTICLE

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION-A REVIEW

K. Bhavya Sri[1]*, Shaheen Banu[1], Mogili Sumakanth[2]

[1]Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad, India.

[2]Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad, India.

***Corresponding Author: K. Bhavya Sri**

Abstract: The reverse transcription polymerase chain response is straightforward, powerful, and exquisite innovation in the discipline of molecular biology. It helps in determining the gene expression and amplifying the gene of interest in numerous fields used to locate and quantify the quantity of a given DNA collection. It entails three steps: reverse transcription, PCR amplification, and electrophoresis. Among PCR, the maximum commonly used PCR technique is reverse transcription polymerase chain reaction abbreviated as RT-PCR. it is way utilized in molecular biology & genetic research that allows the detection & quantification of mRNA. in this, RNA template is first converted into a complementary DNA by use of a reverse transcriptase enzyme and is further used for exponential amplification the use of the PCR method. Many genes are recognized to have their own promoter regions that inform cells where to begin transcribing the unique gene. RT-PCR first starts by using the usage of reverse transcriptase enzyme to make DNA out of RNA that has been extracted from cells or tissue samples. The RNA is in the shape of a polymer. The opposite transcriptase copies the RNA into an open-ended DNA strand, preventing it when it encounters a unique non-coding location known as a "poly-A tail" for each 20 to 23 nucleotides. The PCR method then begins by blending the newly made DNA with known primers to expand or reflect a selected piece of DNA. The pattern is then heated and cooled more than one instance, which causes the DNA to split into exclusive string sizes, brief and long. the quick string represents the newly copied RNA that can be visible as amplification, while the longer strand represents each the amplified RNA and all other non-amplified DNA portions collectively. The difference in the duration of DNA strands is what offers reverse transcriptase PCR its name. RT-PCR approach is performed in a thermocycler for the huge production of genetic fabric. It includes several wells, thermos regulators, a detector, and a gadget hooked up with appropriate software.

Keywords: *Amplification, Thermocycler, Gene expression, cDNA, Molecular biology, RT-PCR, Template.*

INTRODUCTION

- It is an in-vitro approach this is designed to allow selective amplification of a selected target DNA series inside a heterogeneous series of DNA sequences[1].
- There have been a number of key developments in molecular biology [1], however polymerase chain reaction has impacted the maximum in the latest years.

Records

- In 1983, Kary Mullis, even operating with colleagues in Cetus employer, advanced the idea of PCR.
- In 1985, PCR became great innovation to the medical community by way of Kary Mullis and in turn rewarded 10,000 and was presented the noble prize.

Table 1: Components of PCR

Prerequisites of PCR
Oligonucleotide Primer
DNA template
Divalent cation
Taq polymerase
Buffer solution
Deoxynucleotide triphosphate (dNTPs).

The technique of PCR entails repeated cycles which results in amplification of goal DNA. This procedure is carried out in a thermocycler.
each cycle has 3 stages.

DENATURATION

it is also known as the heating step. The DNA molecules are first of all heated at 94℃ for 1 min, to break the bonds among strands of double-stranded DNA [2]. These consequences are inside the formation of separate DNA strands. The template is acquired.

ANNEALING

It is called the cooling step, the temperature is diminished to 54℃ and the template is heated. Hybridization of primer takes place to the template at the 3 and 5 ends. A primer is a short nucleic acid sequence that affects the initiation of DNA synthesis. The length of the GC content must be enough for stable binding with a template [3].

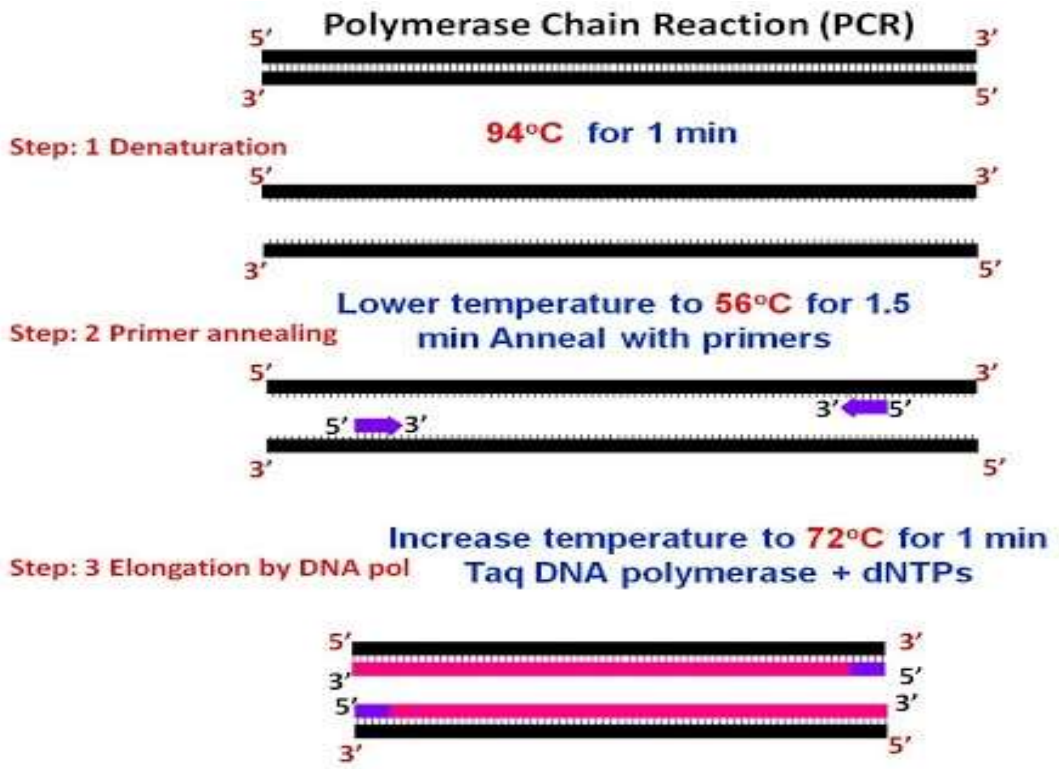


Figure1: Steps involved in PCR

EXTENSION

The reaction aggregate is heated to 72°C in this phase. The primer's elongation begins at this point. Taq polymerase, the elongation-promoting enzyme, is obtained from Thermus aquaticus, a thermostable bacterium. It is resistant to a wide range of temperatures [4,5]. Deoxynucleotide triphosphates (dNTPs) corresponding to template nucleotides are added by Taq polymerase [5].

This leads to the Extension of the new strand [5]. To provide millions of copies of the target DNA or gene of interest, the stages are repeated 35-40 times.

The amplified gene is preserved and kept between 4 and 15 degrees Celsius during the hold stage in order to avoid denaturation [6].

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

A method employed in laboratories to replicate a certain genetic series in large quantities for assessment. Reverse transcriptase is an enzyme that it employs to convert a certain RNA strand into a corresponding DNA strand [7]. The enzyme known as DNA polymerase is subsequently used to amplify this fragment of DNA.

1977 was its introduction. In the process of researching how viruses replicate genetic material, a scientist discovered the reverse transcriptase enzyme [7]. RT-PCR creates complementary DNA [cDNA] transcripts from RNA [7] in order to qualitatively detect gene expression. In molecular biology, this approach is commonly used to find RNA Expression.

PRINCIPLES OF RT-PCR

The study of gene expression has been fundamentally changed by the use of RT-PCR for RNA transcript detection in the following significant ways:

- Made it theoretically possible to detect transcripts of almost any gene.
- Made sample amplification possible and removed the necessity for a large amount of starting material when doing northern blot analyses.
- Allows for RNA degradation to occur as long as the primer-spanning RNA is intact.

One Step

Reverse transcriptase enzyme, primers, DNTPs, RNA, and buffer reagents can all be combined in a single tube before the reaction is started in order to perform RT-PCR in a single step [8]. One-step RT-PCR reduces the chance of contamination while providing ease of use and simplicity. Unlike in two-step, the generated cDNA cannot be utilised to identify numerous messages from a single RNA sample.

Two Steps RT PCR

RT-PCR involves two steps: RT REACTION: Complementary DNA is synthesised at this phase. One tube is filled with each reaction mixture [9]. Reverse transcriptase, mRNA, buffer, dNTPs, and primers are all included in this. RT AMPLIFICATION: Following the synthesis of cDNA, further amplification occurs, producing millions of copies of DNA amplification with one or more gene-specific primers [10].

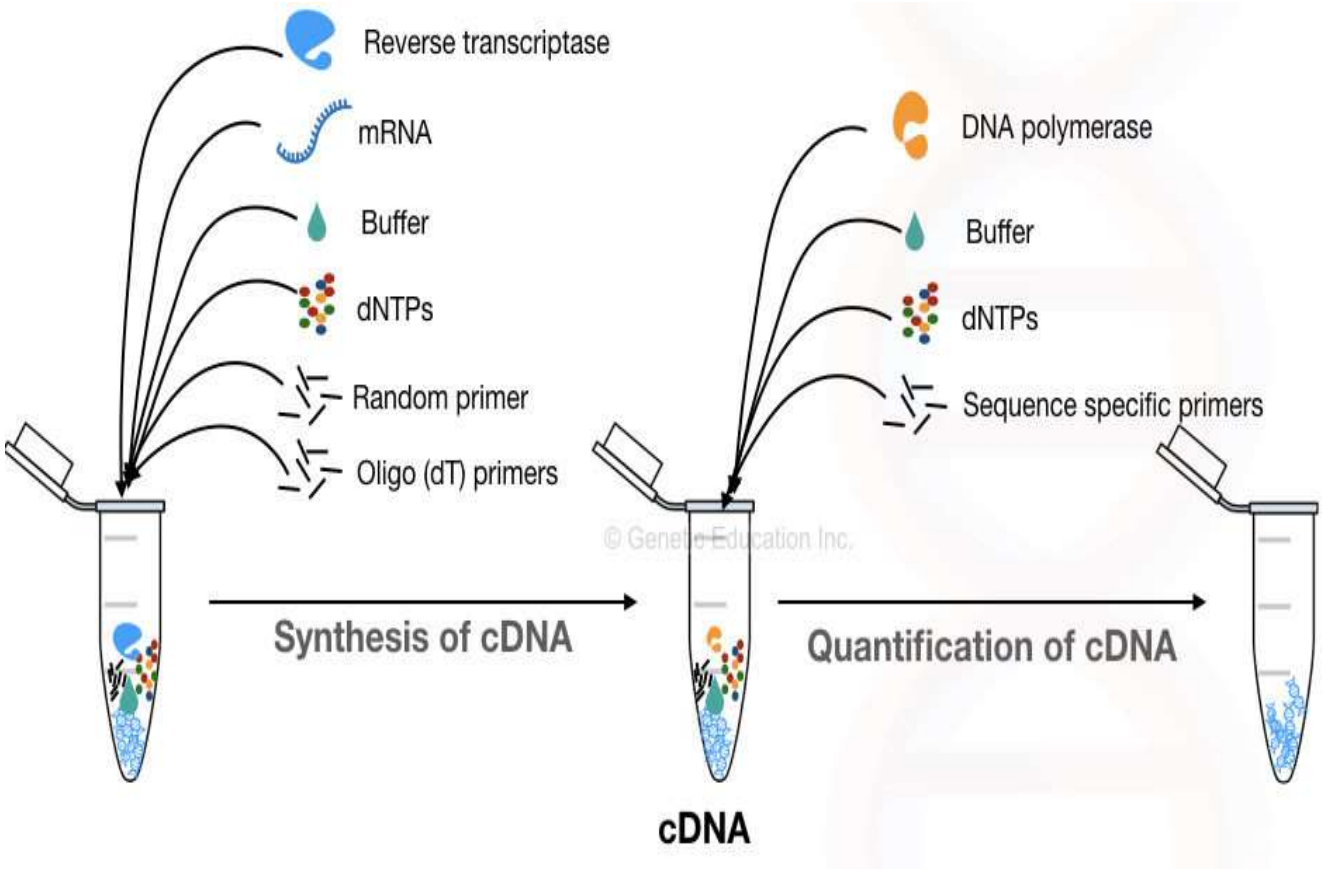
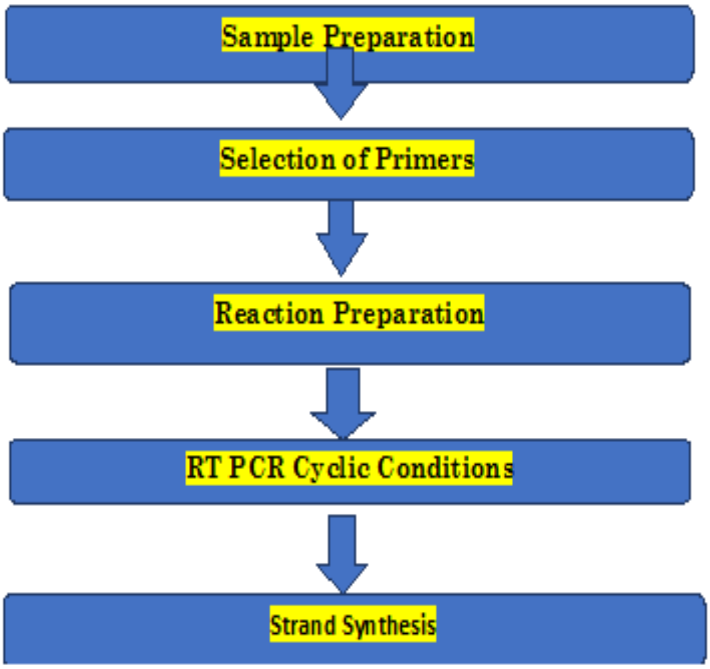


Figure 2: Two step RT-PCR

Steps Involved in RT PCR



Sample Preparation

Start with a microbe culture where the genetic material is RNA. Next, isolate genetic material by separating the cells, and then extract the RNA [11]. The use of RNA extraction rather than DNA extraction for RT-PCR is reminded. Use ready-to-use RNA extraction kits to extract the RNA; they work better and provide a higher yield. Since RNase is found on every viable surface in a lab [11], caution should be exercised when extracting material. RNase is an enzyme that cleaves RNA.

Selection of Primers

To start the chain reaction's elongation, primers are added. They constitute the opening order. In PCR, primer design and selection are crucial steps. Every standard primer requirement should be adhered to in the primer design. Three kinds of primers are required for the process.

- Random primers
- Oligo(dT) primers
- Sequence-specific primer

Random Primers

These are brief, single-stranded base pair sequences made up of hexamers or octamers. The RNA [12] is bound by the random primer at a completely complementary random position.

All forms of RNA, including tRNA, rRNA, and mRNA, can be bound by random primers, which then create cDNA. It is especially important for templates with large secondary structures [13]. High yield cDNA is produced using random hexamer.

Oligo Primer

The oligo (dT) primers have a remarkable mRNA amplification design. The oligo primers exclusively bind to poly-A tail of mRNA [13] because we know that the mRNA contains a poly-A tail. It can therefore fully amplify the mRNA into cDNA. The oligo primers are 12-18 nucleotides long single-stranded DNA with an extra nucleotide at the 3′ end to establish the binding. It can even amplify tiny bits of mRNA. However, because tRNAs, rRNAs, and microRNAs lack the poly-A tail, the oligo primers are only able to synthesis mRNA.

Cyclic Conditions for RT-PCR

Since denaturation is carried out in a single-stranded RNA, it will not occur here. The primer annealing process starts the PCR reaction. After the primer has completed its initial binding to the template RNA, the reaction is cooled to 4°C to ensure correct binding [14]. After the third stage of enzyme deactivation, the second step begins the creation of new strands. The figure illustrates the various RT-PCR steps:

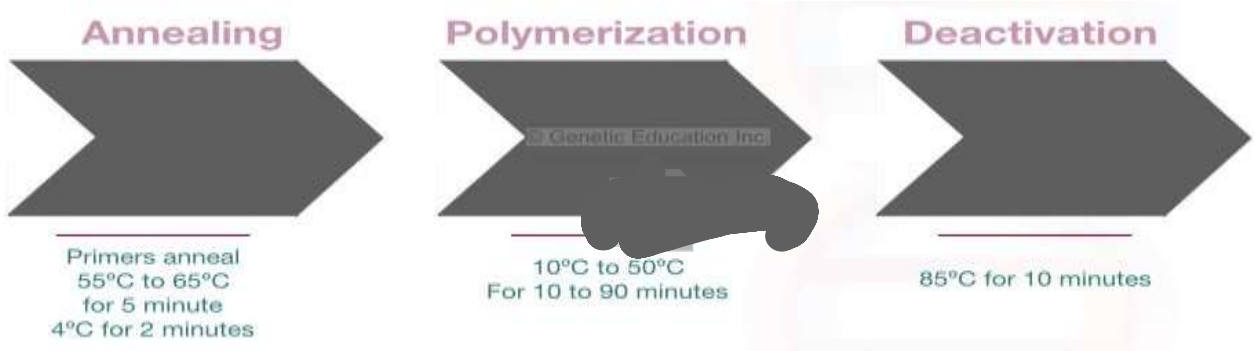


Figure 3: Steps of RT-PCR

Strand Synthesis

- An essential part of the whole RT-PCR procedure is the production of DNA. The synthesis of cDNA is achieved by combining DNTPs with ss RNA and a primer. This process is divided into two stages: first-strand cDNA synthesis and second-strand DNA synthesis [15].
- First-strand cDNA synthesis occurs when reverse transcriptase enzyme amplifies cDNA from single-stranded RNA.
- The RNase activity of reverse transcriptase enzyme breaks the RNA from the RNA-cDNA hybrid into smaller pieces.
- The RNase H action now causes the DNA polymerase to fill in the nicks. Following synthesis, enzyme ligase ligates nicks between neighboring DNA fragments [16].

Advantages of Reverse Transcription PCR

- Qualitative and quantitative Analysis.
- Gene expression studies.
- RNA is amplified directly & the concentration is determined; agarose gel electrophoresis & other similar procedures are not needed.

- The process is quick, easy to follow, economical, & straightforward.
- Sensitivity & specificity.

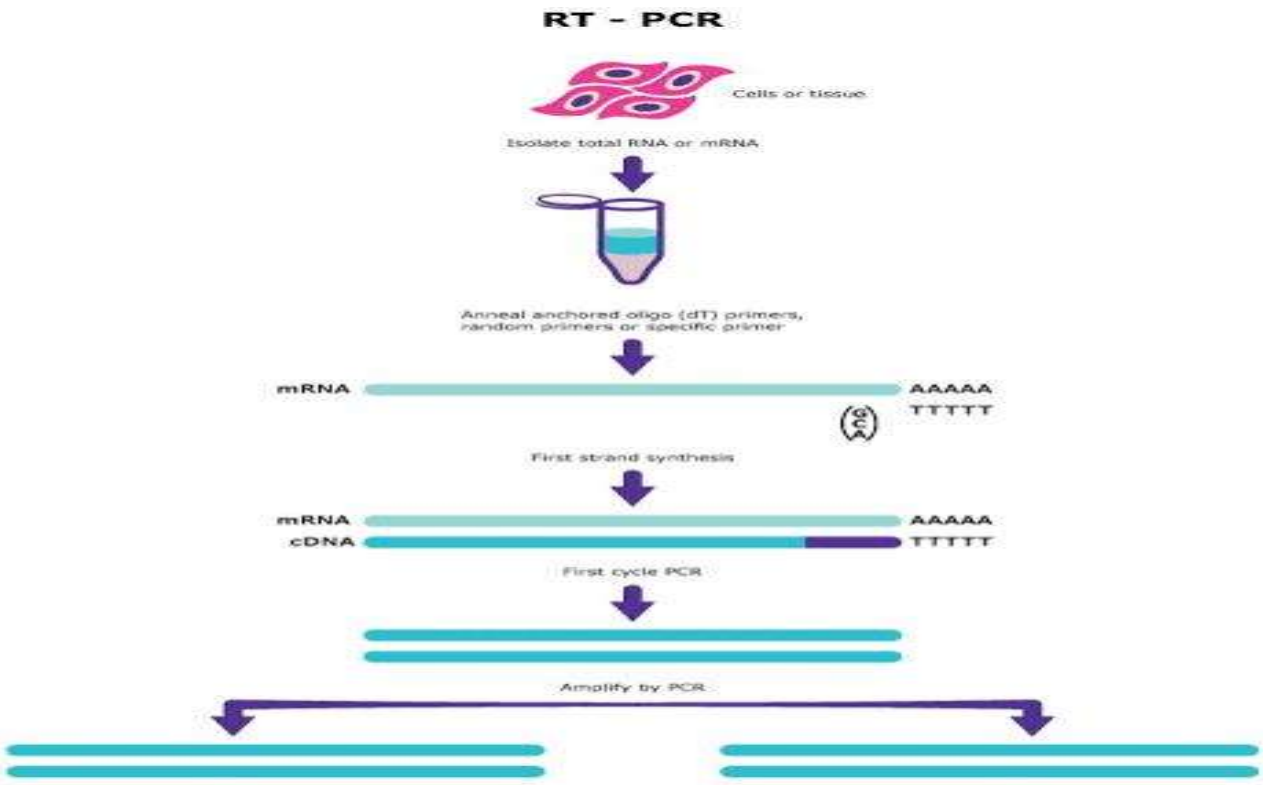


Figure 4: Amplification in RT-PCR

Disadvantages

- Due to the high sensitivity of this approach, even minute amounts of contamination can provide inaccurate findings.
- The assay procedure employs the technology.
- To conduct and create new tests, a great deal of experience and knowledge are needed.

INSTRUMENTATION

A laboratory tool used for PCR analysis is a thermocycler, often known as a PCR machine. The tubes holding the PCR reaction mixtures are put in holes in a thermally insulated block that is part of this apparatus[17].Hot bonnet, a heated plate that presses on the reaction tube lids, is a feature of thermal cyclers [17]. The cycler then raises & decreases temperature of block in discrete, pre-programmed steps. This eliminates the need for PCR oil by stopping water from reaction mixtures from condensing inside the lids.

Figure 5: Thermocycler

Reaction Module

For every cycle of DNA amplification, samples & reaction mixture are precisely heated & chilled to encourage denaturation, annealing, and subsequently polymerase-mediated extension [18].

Temperature Control

- **Peltier**: Itemploys a solid-state active heat pump, which requires the use of electrical energy 18 to move heat across a temperature gradient. The ability to create a temperature gradient with Peltier blocks is a very helpful feature that allows for one run of assay annealing step optimization.
- **Heated and cooled air:** This kind of device circulates air at a specific temperature for predetermined amounts of time, as needed for PCR [18], through a chamber in which tubes are suspended.
- **Formats:** There are various formats available for reaction blocks, but the most popular one is 96-well block with reactions volume ranging from 1 to 125 µl. Blocks with 384 wells are used for measuring samples in nanolitres and picolitres [18].

Optical Detection System

By measuring the fluorescence intensity of each PCR reaction, a target's presence in the sample can be ascertained in the presence of a fluorescent reporter, such as a DNA-binding dye or labeled probe.

Light Sources

Light-emitting Diodes (LEDs): These can be placed in a stationary array to excite the emitting rays in several wells simultaneously, or they can be placed individually or in groups within a shuttle mechanism that is positioned above each well to illuminate each one independently [19].

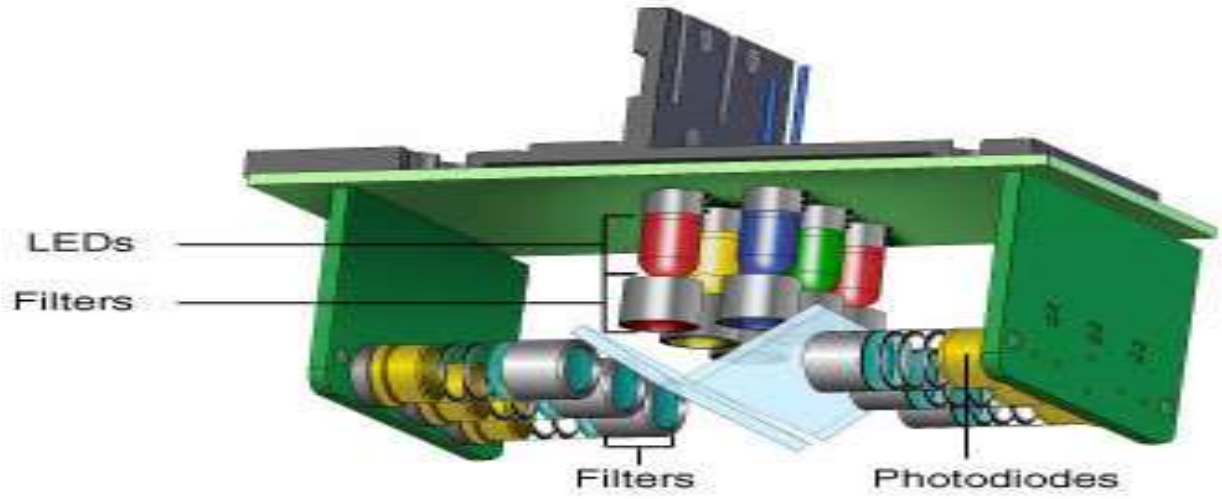


Figure 6: Overview of LEDs

Halogen Lamp: Broad-spectrum white light is emitted by this light source and subsequently filtered to excite particular fluorophores.

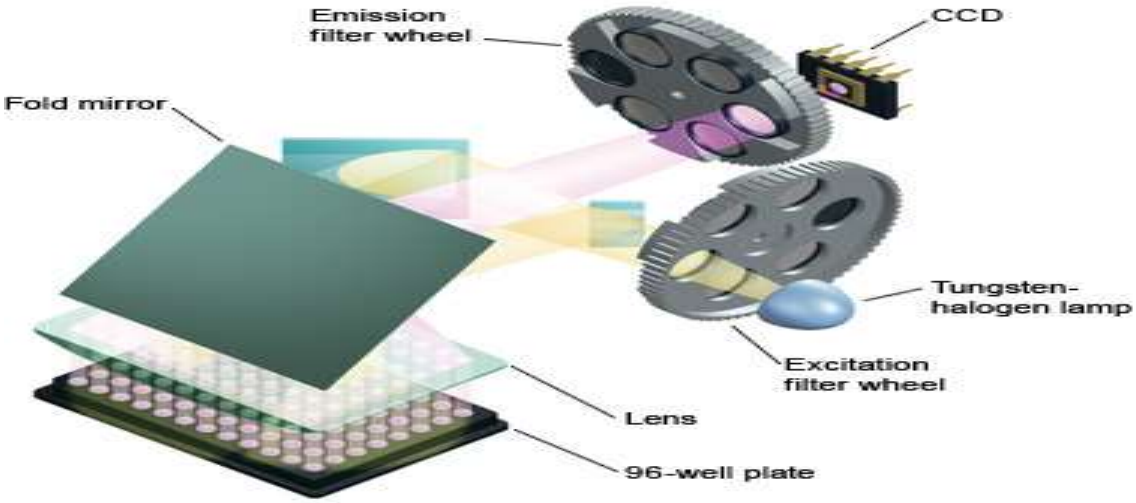


Figure 7: Halogen lamp with wells

Laser: This produces intense light with a limited bandwidth, making it only useful for gathering information from a select group of fluorophores and restricting its application in multiplexing [19].

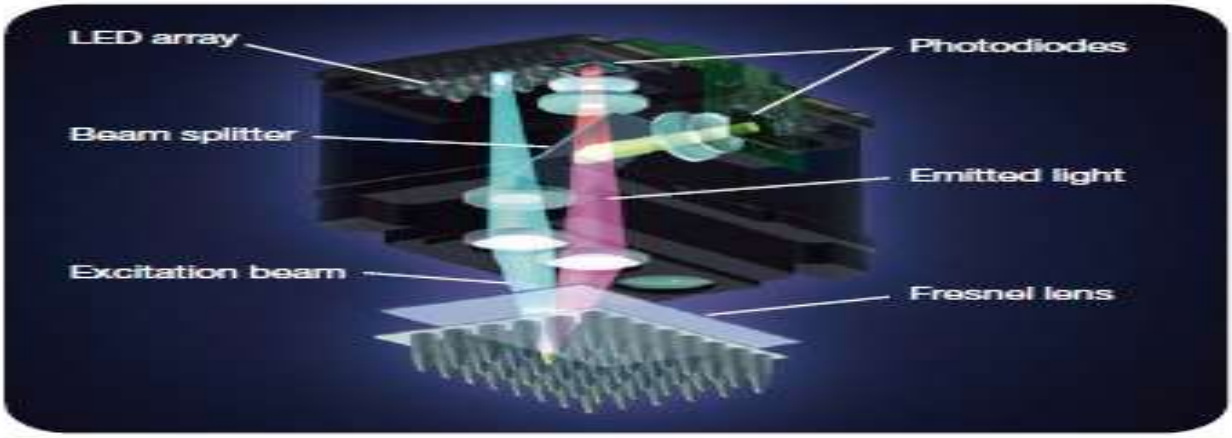


Figure 8: Laser emission

Detectors

A stationary gadget or a moving one can detect light from excited fluorophores. The PCR apparatus uses a variety of detectors. One kind of photodetector that produces a current flow in response to light exposure is the photodiode [19]. These can be fairly small in size and have a broad spectral range and low failure rates. A charge-coupled device creates digital data from the light it detects. The resolution can be used to determine quality of image that is obtained. Typically, CCDs are employed to take an image of reaction plate, which instrument software subsequently uses to interpret its contents. An incident light's ability to produce electricity is multiplied by a photomultiplier tube.

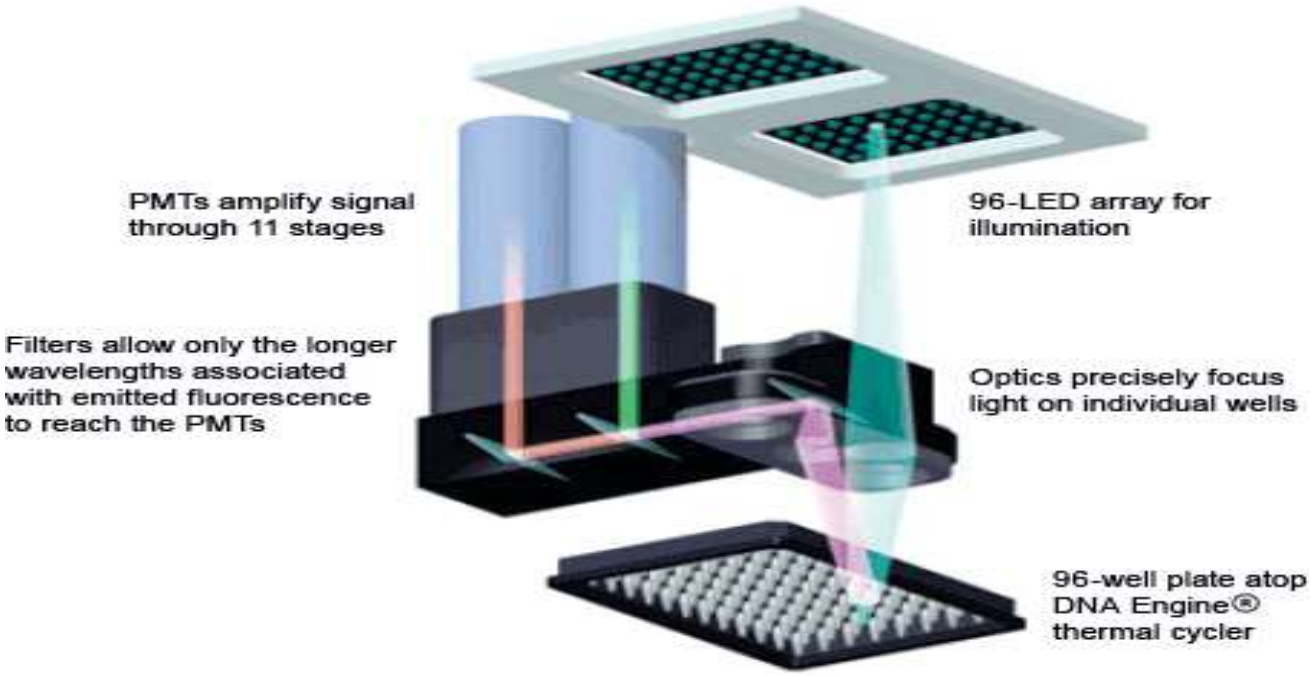


Figure 9: Detector

Instrument Software: Typically, PCR detection devices are operated by an associated computer that runs specialized software to start & stop runs & then make it easier to comprehend the data. The amount of fluorescence present in PCR reactions can be measured using a wide range of optical systems that combine light sources, filters, and detectors [20]. The main parts of real-time instrument software are as follows:

Protocol System: The phases at which data are to be collected, the number of repeated cycles, and the denaturation, annealing, and extension parameters are all provided.

Plate Configuration: Each well's contents are identified to enable accurate data analysis after collection [20]. The scan mode facilitates the selection of a certain channel.

Data Gathering: Each well's reaction mixture's fluorescence intensity is measured and plotted against the reaction cycle. Real-time monitoring of amplification is possible for a given gene expression [21].

Data reasoning: In order to compare the data acquired from several wells, a baseline is automatically produced once a run is finished, or it can be manually specified and applied to each sample. Next, for each well, a quantification cycle (Cq) is established using regression analysis or the location of single threshold line [21].

APPLICATIONS OF RT-PCR

Gene Expression Quantification: The programme is used to graph amplification curves in order to help identify the "cycle time" at which fluorescence approaches a threshold. The amount of precise nucleic acid sequence present in the original sample is negatively correlated with this CT value. In order to quantify cDNA and ascertain gene expression, one uses the CT value.

Identification of Mutations by Melting Curve Analysis: After PCR is finished, the

hybridization probes are attached to the amplicon in tandem, allowing energy to transfer from the donor to the acceptor fluorophore, which emits a signal. This process is done automatically to detect mutations in the sequence, and fluorescence measurement hybridization probes are frequently used. Allelic discrimination, or genotyping individuals or experimental organisms, is accomplished using this kind of study.

Biomedical Research: RT-PCR can be used to genotype knockouts and assess the effectiveness of gene & delivery strategies in cell culture systems or animals. Allelic discrimination in PCR allows for the identification of SNPs in populations that may predispose people to specific diseases, which helps with epidemiological research.

Molecular Diagnosis: PCR is able to quantify the course of a disease and the effectiveness of antiviral treatments. Melting curve mutation analysis facilitates both individual and epidemiological investigations of viral coinfections.

It aids in lowering the usage of antibiotics with a broad spectrum, which could promote the formation of strains resistant to antibiotics. Neisseria gonorrhoeae, Legionella pneumophila, Listeria monocytogenes, and Mycobacterium tuberculosis have all been identified with RT PCR.

Counting Bacterial, Viral, or Fungal Loads: Certain sequences can be identified from a complicated combination of DNA using RT-PCR. This makes it helpful for figuring out whether a sample contains pathogen-specific or various unique sequences, as well as its quantity.

In forensic Sciences: Additionally, forensics labs use PCR, which is particularly helpful because it only requires a very small amount of original DNA-for instance, enough DNA can be extracted from a single hair or a droplet of blood.

Analysing viruses: PCR is used to examine clinical specimens for presence of infectious agents such as HIV, hepatitis, malaria, anthrax, and other illnesses.

Human genome project: PCR is a crucial step in the cloning process that makes it possible to create large amounts of pure DNA from tiny amounts of template strand and to study a particular gene.

The Human Genome Project (HGP), which aims to determine the sequence of the 3 billion base pairs that make up the human genome, is heavily dependent on PCR.

DNA microarray results validation: Due to the accuracy of RT PCR, a lot of researchers confirm and collaborate with the findings of imprinted DNA microarrays as well as oligonucleotide arrays (like the Affymetrix Gene Chip) using relative or absolute measurement of gene expression.

RT-PCR test for COVID-19: The method uses reverse transcription & amplification to quantify the quantity of virus load in a sample. The performer began the method by transcribing the RNA into DNA in reverse. Using a ready-to-use COVID-19 testing kit, which typically contains all the materials required for RT-PCR, it prepares the PCR reaction. Approximately 38 to 42 amplification cycles are specified in the cycling conditions. The COVID-19 RT PCR's CT value provides information on the viral load. Lower the CT value, the higher the load, and vice versa.

Figure: 10 Covid kit

Genetic Disease Diagnosis: PCR is used to analyse mutations that arise in a variety of hereditary illnesses, such as muscular dystrophy, phenylketonuria, sickle cell anaemia, and cystic fibrosis.

Cancer detection: PCR can forecast a patient's response to treatment or resistance to it, as well as provide information about the patient's prognosis. PCR is used to find minor mutations in certain genes, which are characteristic of many malignancies.

In Evolutionary Biology: PCR has been employed in evolutionary biology to find and investigate links between them.

- Ancient human patterns of migration in anthropology.
- Ancient human race in archaeology.
- By palaeontologists - To amplify genetic material from extinct animals or millions of years' worth of cryopreserved remains.

Table 2: PCR VS RT-PCR

PCR	Reverse Transcrition PCR
Through the use of PCR, a gene segment can be amplified and millions of copies of DNA sequence can be produced.	In molecular biology, RT-PCR is a type of PCR used to measure gene expression.
The three phases of PCR are denaturation, annealing, and extension.	After reverse transcription, PCR is done. The denaturation stage is absent.
A template is a molecule of double-stranded DNA.	The template utilised is a single-stranded RNA molecule.
The enzyme in question is DNA polymerase.	Enzymes such as DNA polymerase and reverse transcriptase are employed.
Forward and reverse annealing takes place by attaching the primer from both the ends of strand.	Only reserve annealing of primer takes place.
It is comparatively less sensitive than RT-PCR.	This method is highly sensitive.
It is utilized in DNA sequencing, DNA cloning, functional investigation of genes, diagnosis and tracking of genetic disorders, and amplification of ancient DNA.	It is employed in forensic sciences, cancer diagnosis, viral analysis, evolutionary biology, quantification of gene expression, and mutation detection [22].

CONCLUSION

Two crucial molecular biology procedures are PCR and RT-PCR. Automated methods called PCR and RT-PCR may both exponentially increase the quantity of copies of target genetic sequence, which is determined by forward & reverse primers. Double-stranded DNA is the template used in PCR.

It is possible to perform PCR, DNA cloning, DNA sequencing, and gene functional analysis. RT-PCR amplifies RNA to assess gene expression in specific tissue. The templates utilized in each reaction and applications they are used for are the primary distinctions between PCR & RT-PCR.

ACKNOWLEDGMENT

We are thankful to our Principal, Prof. M. Sumakanth of RBVRR Women's College of Pharmacy for giving us this opportunity to perform this research work.

REFERENCES

1. Polymerase Chain Reaction (PCR): National Center for Biotechnology Information. U.S. National Library of Medicine, 01 June 2017.
2. Polymerase Chain Reaction (or PCR), Cloning and Molecular Analysis of genes, 01 June 2017.
3. Biolabs, New England. "CDNA Synthesis and RT-PCR." CDNA Synthesis and RT-PCR | NEB, 01 June 2017.
4. Rapley R, Theophilus BDM, Bevan IS and Walker WR: Fundamentals of the polymerase chain reaction: A future in clinical diagnostics. Med Lab Sci 1992; 49(2):119-128.
5. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific Enzymatic Amplification of DNA *in vitro*: The polymerase chain reaction. In: cold spring

Harbour Symposia on Quantitative Biology 1986;51(1):263-273.

6. Pelt-Verkuil E, Belkum A, John P. A brief comparison between in vivo DNA replication and in vitro PCR amplification. Principles and Technical Aspects of PCR Amplification. Netherlands: Springer; 2008. pp. 9-15
7. Lawyer FC, Stoffel S, Saiki RK, Myambo K, Drummond R, Gelfand DH. Isolation, characterization, and expression in Escherichia coli of the DNA polymerase gene from Thermus aquaticus. Biological Chemistry. 1989;264:6427-6437
8. Marcela AAV, Rafael LG, Lucas ACB, Paulo RE, Alessandra ATC, Sergio C. Principles and Applications of Polymerase Chain Reaction in Medical Diagnostic Fields: A Review. Brazilian Journal of Microbiology. 2009;40:1-11
9. Maecela Agne, Rafael Lima, Licas Andre, Paulo Roberto, Alessandria de Tavaras and Sergio Crovela. Principles and Applications of Polymerase chain Reaction in Medical Diagnostic fields- A Review. Brazilian Journal of Microbiology. 2009;40;1-11.
10. Tahmiur Rahman MD, Muhammed Salahuddin, Razia Sultana, Arumina Moue and Muntahina Setu. Polymerase Chain Reaction- A Short Review. AKMMC. 2013;4(1):30-36.
11. Joshi M and Deshpande JD. Polymerase Chain Reaction-Methods, Principles and Applications. International Journal of Biomedical Research. 2010;1(5):81-97.
12. Tabitha M. The Polymerase Chain Reaction Breakthroughs in Bioscience- A Series of articles for general audiences.
13. Waleed A Ibrahim, Abdul Ghany A, Soad A Nasef and Hatem ME. Comparative Study on the Use of RT-PCR and Standard Isolation Techniques for the Detection of Salmonella in Broiler Chicks. International Journal of Veterinary Sciences and Medicine. 2014;2:67.
14. Pusterla N, Madiga JE and Leutenegger CM. RT-PCR A Novel Molecular Diagnostic Tool for Equine Infectious diseases. Journal of Veterinary Medicine. 2006; (20):3-12.
15. Kary Mullis B. The Usual Origin of Polymerase Chain Reaction. Scientific American Journal. 1990. 12. Lilit Gariyan and Nidhi Avashia. Research Techniques Made Simple PCR. Journal of Invest Dermatol. 2013; 133(3):6.
16. Pablo Perez-Pinera, Manuel Menendez Jose and Antonio Vega. Detection of DNA Sequences of Using PCR Based Approach. Electronic Journal of Biotechnology. 2006; 9(5):22-25.
17. Bria D Mariani, Daniel S Martin, Antonio F Chen, Haruyo Yagi, Shaldon S Lin, and Rocky S Taun. PCR-Molecular Diagnostic Technology for Monitoring Chronic Osteomyelitis. Journal of Experimental Orthopaedics. 2014; 19.
18. Ying Wang, Zhanli Wang, Chunfang Liu, Anma and Hui Yu. PCR-based Assays for the Diagnosis of Human Brucellosis. Annuals of Clinical Microbiology and Antimicrobials. 2014; 13:31.
19. Masool Majaz, Parvaiz Hassan, Vijeshwar Verma and Zafar Reshi. Inhibition of Polymerase Chain Reaction by Lithium chloride. International Journal of Life Sciences and Pharma Research. 2012; 2(4).
20. Margaret F Docker, Robert H Delvin, Jon Richard and Khattra J. Sensitive and Specific PCR- Assay Detection of Loma Salmonae (Microsporea). Inter search. 1997; 29:41-48.
21. Dangi SK, Singh AP and Dangi SS. PCR Amplification Cloning of Immunogenic Protein and Dependent Hydroxybutyracryl Dehydrogenase Gene of Clostridium Chauvoei. Veterinary World. 2015;7(10):848-51.
22. Thoraia Shinawi. Lab Manual of Molecular Biology Lab. M. Wink, Heidelberg; Bio forum Extra- Prague. 1994; 5-17.